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FACULTY OF GRADUATE STUDIES AND RESEARCH

(Signed).....Jo Anne Garrod.....

PERMANENT ADDRESS:
RR 2 Wallenstein Ont
.....
MAILING ADDRESS c/o Rothkranz
RR 1 Queensville Ont

DATED..Sept. 1.....1973

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THE UNIVERSITY OF ALBERTA

THE EFFECTS OF EXERCISE AND THREE FEEDING PATTERNS ON THE EPIDIDYMAL FAT CELL OF THE RAT

By

© Jo Anne Lillian Garrod

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effects of Exercise and Three Feeding Patterns on the Epididymal Fat Cell of the Rat" submitted by Jo Anne Lillian Garrod in partial fulfilment of the requirements for the degree of Master of Science.

.....
Supervisor

.....

.....

Date June 21/73.

ABSTRACT

The effects of 10 weeks endurance training, three feeding patterns, exhaustive exercise, and epididymal lipidectomy were investigated in 120 male Wistar rats aged 8 weeks. The rats were run on a treadmill at one mile per hour, for one hour, five days a week. Pair feeding, paired weighting and ad libitum feeding were the feeding regimens used. Epididymal fat pad lipid content was determined and suspensions of fixed, isolated cells were employed for determination of fat cell size. Total fat cell number was calculated indirectly from the diameter and lipid content data. No significant effects were produced by any of the feeding patterns. Exercise reduced the total fat cell complement of the epididymal fat pad and running to exhaustion significantly reduced cell size. The lipid content of the cells was also significantly reduced by exercise. The lipidectomized rats demonstrated adipose tissue regeneration that was subject to the same effects of exercise as normal adipose tissue.

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CHAPTER I

INTRODUCTION

In 1964 Rodbell (Rodbell, 1964) discovered an effective, practical, method for isolating viable fat cells. The research which followed this discovery has recently caused the recognition of adipose tissue as an important embryologically differentiated organ. It is known that the tissue has an active thermogenic function (Himms-Hagen, 1971) and is a major center for the provision of oxidative energy during prolonged exercise (Cobb and Johnson, 1963). Adipose tissue also responds actively to stimuli such as caloric intake, diet, cold acclimation and mental and emotional strain (Wertheimer, 1965). This activity is a result of rich innervation and vascularization which enables precise control of lipid deposition, mobilization and turnover.

The epididymal fat pad of the rat is metabolically active, readily isolated and can be totally removed from a living or dead rat by simple dissection. Though the metabolic responses of these tissues are qualitatively and quantitatively unlike those of humans, the morphology and growth patterns have demonstrated a high degree of similarity (Bjorntorp and Ostman, 1971). Thus this animal provides an excellent source for adipocyte experimentation.

Recent experimentation has shown that when various cell sizes are compared for their ability to synthesize lipid from glucose (Bjorntorp and Sjostrom, 1972) to esterify labelled fatty acids in the presence of glucose (Bjorntorp and Sjostrom, 1972) or to respond to insulin (Bjorntorp and Sjostrom, 1971), differences are observed.

The larger cells demonstrated a greater absolute response per cell but a lesser capacity per unit weight of lipid. This has been found to be true for animals of different ages (Austin and Foxman, 1969) and animals and patients in different states of obesity (Knittle and Hirsch, 1968). The relationship also holds as well for the same subjects before and after weight loss (Bjorntorp and Sjostrom, 1971).

The response of the cells to lipolytic hormones showed similar patterns. The larger cells were found to be less sensitive to lipolytic hormones (corticosteroids, norepinephrine and epinephrine) even though the release of free fatty acid per cell was greater (Zinder and Shapiro, 1971; Bjorntorp and Sjostrom, 1972). These comparisons were based on the relationship between surface area and lipid content.

There are indications that larger cells can deposit and mobilize stored fat more quickly although relatively the response of the larger cells is considerably less. Further studies of lipid deposition and mobilization in fat cells have illustrated that there could be definite efficiency advantages in maintaining cell size within functional limits. (Knittle and Hirsch, 1968; Greenwood, Johnson and Hirsch, Bjorntorp and Sjostrom, 1971; Bjorntorp and Sjostrom, 1972).

The response of fat cell size to physical training, dietary manipulations, cold exposure and age has been studied in animals. All of these variables significantly affect adipocyte size (Palmer and Tipton, 1972; Knittle and Hirsch, 1968; Booth, Booth and Taylor, 1973; Oscai, Spirakis, Wolff and Beck, 1972). Starvation, cold exposure and physical training, which have in common that they create an energy requirement, were all associated with reductions in fat cell size.

The number of cells in adipose tissue is subject to less variability. As a rule the tissues and organs of the body acquire their full adult complement early in life. However, studies by Bjorntorp and Sjostrom (1972) and Hirsch and Han (1969) have demonstrated that certain extreme states of obesity are associated with a much expanded cell complement.

The regenerating ability of epididymal fat pads has not been subjected to such extensive investigation. In a study by Taylor and McBean - Hopkins (1971), it was demonstrated that regeneration of lipidectomized fat does occur. However, cellular hypertrophy is more evident in control animals whereas cellular hyperplasia is dominant in the animal receiving regular exercise.

Rationale Behind The Study

From the foregoing discussion, it would appear that a large number of small cells would be advantageous in an animal which was required to store and mobilize, rapidly and frequently, large amounts of lipid. Such would be the case in rats undergoing daily endurance training, since lipids provide the major source of energy for working muscles (Issekutz and Paul, 1966).

The adult obesity state in man (Hirsch and Knittle, 1970) and animals (Johnson and Hirsch, 1972) appears to be associated with hyperplasia as well as hypertrophy of fat cells. Animal studies (Johnson and Hirsch, 1972) have clearly indicated that some obesities due to enlarged cell complements are genetic in nature, but permanent cell number changes may be induced in genetically normal and abnormal

rats by a severe metabolic or nutritional stimulus applied very early in life (Knittle and Hirsch, 1973; and Oscai, Spirakis, Wolff and Beck, 1972). A decreased adult cell number has resulted from undernutrition in pre-weaning rats, (Knittle and Hirsch, 1968) whereas cold exposure begun at four weeks of age has produced the opposite effect (Therriault, Hubbard & Mellin, 1969). Oscai, Spirakis, Wolff and Beck (1972) have demonstrated that exercise in addition to food nutrition (in the form of paired weighing) in early life is effective in reducing the rate of accumulation of cells in the epididymal fat pads. Recently, Booth, Booth and Taylor (1973) have illustrated that training does not significantly affect cell number in rats seven to sixteen weeks of age but was effective in maintaining cell volume at a level approximately half that found in sedentary rats. Regular exercise has also demonstrated a positive effect on cellular proliferation of lipidectomized Wistar rats. (Taylor and McBean - Hopkins, 1971).

Statement of Problem

This study was designed to investigate the effects of regular endurance training, pair feeding, paired weighting and ad libitum feeding patterns on fat cell size, lipid content and the total number of fat cells in the epididymal fat pads of the post-pubertal Wistar rats. The effect of unilateral and bilateral lipidectomy on these physiological parameters was also considered as a sub problem to this study.

Limitations Of The Study

1. This study was confined to the epididymal fat pad of the male Wistar rat seven - twenty-four weeks of age, thus results may not be extrapolated to females or other species.
2. The measurement and control of food intake was lacking in precision since it was not possible to estimate what percentage of food was spilled outside the cages.
3. Only one intensity of endurance training was used, and some trauma could have resulted from the presence of the electrical shocking device on the treadmill.

CHAPTER II

REVIEW OF LITERATURE

Effects of Age and Exercise on Fat Cell Number and Size

Fat accumulates in the epididymal fat pad of rats as a result of an increase in fat cell number and fat cell size (Hirsch and Han, 1969). However, it is known that at approximately fifteen weeks of age (Hirsch and Han, 1969) cell number becomes fixed in the depot and only the cell size changes with further increases in adiposity. Recent evidence has shown however, that exercise causes a reduction in the total body content of fat in young growing rats (Crews, Fuge, Oscai, Holloszy and Shank, 1969), Therefore past and on-going research has been directed to determine whether exercise retards the rate at which adipose tissue cells accumulate or enlarge, or both.

Extreme changes in depot size caused by starvation or experimental obesity, induced at approximately the sixth week of life, produced no change in final cell number (Hirsch and Han, 1969). But in other work from Hirsch's lab (Knittle and Hirsch, 1968), it was shown that much earlier nutritional effects can change the adipose cell number of the rat. When rats were nutritionally deprived during the first three weeks of life they were stunted in nearly all aspects of growth and development, and in spite of ad libitum feeding following weaning, the adipose depot remained small, with a low cell number and small cell size.

The fixed number of mature adipocytes, unaffected by extreme and long-term changes in depot size, favors the hypothesis that adipocytes

are formed early in life by a process that is responsive only to very early influences. Once formed, these adipocytes can change size but do not change in total number (Hirsch and Han, 1969). These findings now focus attention on early modification of adipose cellularity as an important feature of metabolic capacity.

Oscai and his associates (Oscai, Spirakis, Wolfe and Beck, 1972), Parizkova and Stankova (1964), and Bjorntorp et al (1971), have reported that training results in smaller fat cells. The methods of these researchers consisted largely of a variety of indirect methods for determining the dimensions of fat cells. However, since Bray (1970) and DiGirolano, Mendlinger and Fertig (1971), have perfected a microscopic technique for measuring cell size, Palmer and Tipton (1973) conducted an investigation to measure directly the influence of chronic exercise on the size and distribution of epididymal fat cells isolated from both normal and hypophysectomized rats. Their results indicate that repeated periods of exercise significantly reduced the size and altered the pattern of distribution of sizes of isolated fat cells.

It was found that the exercise program produced significant biochemical changes in the trained animals. These changes included marked increases in the succinic dehydrogenase activity which supported the findings of Gollnick and Ianuzzo (1972) but which were not of the magnitude which Holloszy (1967) found using a more strenuous exercise program.

Concomittant with the biochemical adaptation of the exercise trained groups, there was significant decrease in adipocyte cell size as reflected by diameter, area and volume (Palmer and Tipton, 1973).

Their findings have helped to confirm the results of Parizkova (1964), Oscai (1972), and Bjorntorp et al (1971), who used indirect methods, hence the reduction in cell size was shown to be related to the energy requirement of the trained groups and not to a decrease in food consumption because 'paired weighting' procedures ensured that the exercise rats were consuming the same amount of food as the control animals.

Since free fatty acids (FFA) are able to supply between 30 and 70 per cent of the total energy requirements during normal exercise (Issekutz, 1966) adipocytes play an important role in the supply of substrate to working muscle. Associated with the increased caloric requirements of exercise, is the increased amounts of circulating hormones capable of eliciting the release of FFA from adipcoytes (Mangaviello and Vaughn, 1972). The increased mobilization of substrate during exercise which reduces fat cell size in trained animals is hypothesized to be related to the similar findings of Parizkova et al (1964) and Goldrick and Williams (1969). These researchers took adipose tissue from trained animals and studying it in vitro found that catecholamines stimulated a greater release of non-esterified FFA in the trained controls than in their sedentary controls. However, it is still unclear whether the training effect imposes a metabolic adaptation.

Similar approaches to this problem involving the effect of exercise on fat cell size have been undertaken by Oscai et al (1972) and Booth, Booth and Taylor (1973). Oscai's exercise program consisted of swimming the rats for 6 hours/day six days a week while the program of

Booth et al (1973) involved treadmill running at one mile per hour for one hour five days a week. In order to control food intake Oscai used 'pair weighting' techniques while Booth used 'pair feeding'. In general the results are the same. That is, the cells of the trained animals are consistantly and significantly smaller than the matched controls and have an increased metabolic capacity.

Only Oscai's study (1972) which dealt with rats aged eight days to seventeen weeks elicited any effect on fat cell number. The total number of cells decreased, but since they also decreased in size the exercised rats had a significantly higher number of cells than the sedentary rats when expressed per gram of adipose tissue.

Feeding Programs

The method pair feeding involving equalization of food intake among a number of pairs of animals, was first applied by Armsby (1921) to study protein requirements. This pattern of feeding merely ensures a balanced diet between two groups of animals so that the effects of another variable may be better observed. This method is relatively uncommon in physiological studies although it has been used by Florence and Quarterman (1972) to investigate the effects of age and feeding pattern on glucose tolerance and plasma free fatty acids in the rat.

The most common feeding pattern used is that of paired weighting, a program of feeding for equal gains in body weight. Palmer and Tipton (1973), and Oscai et al (1972), have used this program to ensure that the animals are of comparable body weights since it has been demonstrated that regularly exercised rats have smaller appetites and weigh less

than their freely eating, non-exercised controls (Stevenson, Box, Feleki and Benton, 1966). This is a necessary control for the study of adipocytes since it has been found that the size of the adipocyte increases as the body weight increases (Johnson and Hirsch, 1972).

Lipid Content of Fat Cells

Lipid content of adipose is easily determined as a carboxyl ester content by the method of Rapport and Alonzo (1955). It has been conclusively shown that in all instances exercise significantly reduces the concentration of lipid as well as the total lipid content of the tissue (Key et al., 1956; Ringite et al., 1964; Grollman and Costello, 1972).

Metabolic Responses of Fat Cells

The liberation of intact fat cells from adipose tissue by collagenase digestion allows the cells to retain their original capacities to synthesize triglyceride, to oxidize glucose and to respond to lipogenic and lipolytic hormones (Rodbell, 1964). Thus it has been possible to conduct studies on the relationship between cell size and metabolism. Cell lipid content, cytoplasmic mass, membrane surface area and enzyme concentrations have all been examined as the size dependent components explaining varying metabolic responses.

Experiments have been conducted on both humans and animals of different ages, and in different states of obesity. In general the results have shown that the larger cells demonstrate a greater absolute response per cell but a lesser capacity per unit weight of lipid.

The metabolic parameters for which this result has been discovered are: the ability to synthesize lipid from glucose (Goldrick and McLoughlin, 1970; Bjorntorp and Karlsson, 1970; Smith, 1971; Bjorntorp and Sjostrom, 1972), to esterify labelled fatty acids in the presence of glucose (Bjorntorp and Sjostrom, 1972), and to respond to insulin (Greenwood, Johnson and Hirsch, 1970: Smith, 1971; Bjorntorp and Sjostrom, 1971). The same relationship between efficiency and cell size was found when large and small cells were obtained from the same patients before and after weight loss (Bjorntorp and Sjostrom, 1971; Salans, Horton and Sims, 1971). The most conclusive evidence however, has come from Bjorntorp and Sjostrom (1972) who have confirmed the results with cells obtained from the same pads of the same subjects at the same time, which were separated into fractions of mean size and compared in terms of metabolic capacity.

Responses elicited by lipolytic hormones have also been compared (Hartman et al., 1972; Bjorntorp and Sjostrom, 1972). The larger cells have been found to be less sensitive to the hormones although the absolute release of FFA per cell was greater. These findings imply that for a given fat cell complement, the larger cells, in fact, deposit and mobilize stored fat more quickly. However, in relation to the total amount of lipid stored the response of the larger cells is considerably less. Thus it has been illustrated that there are various size-dependent components of the cell which determine metabolic responses.

Growth of Fat Cells

Enlargement of the fat cells is accomplished by increases in both the lipid and cytoplasmic compartments. Whereas it has been found that the lipid growth is easily quantifiable, the cytoplasmic growth is not. Most investigators have attempted to measure cellular protein of the cytoplasmic mass, but collagenase, albumin and stromal proteins interfere with the results (Rodbell, 1964a; Goldrick, 1967). Even though different methods have been used; such as measuring cell water and 'lipid free' residual space (Martinsson, 1968), or isotoping collagenase and albumin (Salans and Dougherty, 1971), the results have all been qualitatively similar. The investigators have shown that the ratio of lipid to the cytoplasmic index increased as the cell enlarged.

Surface area also increases as the cell enlarges. Reh has justified (cited by Goldrick, 1967) the assumption that the adipocyte is a sphere *in vivo* thus the surface area to volume ratio of the cell declines as it enlarges. This ratio is considered to be critical to the efficient operation of the biological unit (Goss, 1964) since most of the hormonal receptor sites are believed to be membrane bound. Thus the surface area has taken on particular significance in explaining the metabolic response to cellular enlargement (Zinder and Shapiro, 1971; Bjorntorp and Sjostrom, 1972).

These changes in absolute amounts of lipid, cytoplasm and surface membrane enzymes account for the increased absolute capacity of the large cell to deposit and mobilize fat. However, as the cell enlarges decreases occur in those critical ratios which normally provide

for the control of cellular activity surface area/volume ratio, protein/lipid ratio, enzyme/lipid ratio and the DNA/cytoplasm ratio. Zinder and Shapiro (1971) and Hartman et al. (1971), have proposed a limited capacity of the cell to produce new membrane receptors thus producing hormonal insensitivities and reduced metabolic capacities. However, the quantitative relationships between the critical cell ratios and the metabolic capacity of the cell have not yet been investigated.

Regeneration of Fat Cells

The majority of research at this time indicates that with age, adipose tissue progressively loses the ability to grow by hyperplasia, and this suggests that the mature fat pad growth is primarily due to hypertrophy (Taylor and McBean-Hopkins, 1971). Taylor et al. have conducted two studies (1971, 1972) to investigate the ability of adipose tissue to regenerate. The first study investigated the effects of exercise, bilateral and unilateral lipidectomy on the regeneration of rat fat pads while the second was to investigate the regeneration and the effect it had on the mobilization of FFA during exercise and training. Both studies used the DNA content of the fat pad to measure total regeneration. The lipidectomies were performed while the rats were 6 to 8 weeks of age.

Treadmill training programs have been shown to increase the DNA content of rat adipose tissue (Parizkova, 1966). This has been hypothesized to indicate a change in metabolic activity (Parizkova, 1966), a change in proliferation (Kazdova, 1967), or both (Braun et al., 1968).

Since it is well established that physical activity inhibits the hypertrophy of fat cells (Parizkova, 1966), the finding that the fat pads were relatively the same size after 16 weeks regneration in all lipidectomized animals whether trained or control, and that the trained rats had higher DNA contents of the fat pads, indicates a greater cellularization of the trained animals and greater hypertrophy of the sedentary animals.

Lipid mobilization in plasma and fat depots is stimulated and enhanced by muscular work (Taylor, Booth and McBean, 1972) in the intact fat pads of unilaterally lipidectomized exercisers. However, even though the bilaterally lipidectomized animals of Taylor's (1972) study were able to run as long as the controls the adipose tissue demonstrated complete failure to mobilize FFA or to release (in vitro) FFA. This lack of activity is attributed to incomplete nervous tissue regeneration or inadequate circulation to the reformed fat pads (Taylor, Booth and McBean, 1972).

Thus evidence is avilable that cellular proliferation may not be totally determined prenatally and that under stressful conditions the adipocytes can change in size and number, although the regenerated organ may be a depot and not a functional metabolizing unit.

CHAPTER III

METHODS AND PROCEDURES

One hundred and twenty male Wistar rats, aged eight weeks were used for this study. On arrival the rats weighed approximately 180 g. The animals were placed in 7" x 10" x 7" self-cleaning cages in a room with the temperature controlled at 22.5°C and a twelve-hour light and dark cycle. Twenty-four animals were randomly assigned to the Regeneration Study and the remaining rats comprised the subjects for the Diet and Exercise Study.

Three different feeding patterns were employed in the study. The 'ad libitum' (Group 1) program exercised no restrictions on food intake. These rats were allowed to consume an unlimited quantity of food. To execute the 'pair feeding' (Group 2) program, the amount of food consumed by the exercise rat on day one was measured and given to the matched sedentary rat on day two. This procedure was continued to the end of the program, at which time the sedentary rat was sacrificed one day later to complete the diet regulation. The quantity of food consumed in the 'paired weight' (Group 3) group was determined by the difference in body weight within pairs of exercise and sedentary rats. Thus controlled feeding was utilized to regulate and maintain equality of body weights. This regimen was also carried out until the end of the program, but in this case the sedentary rat and his matched exercised rat were sacrificed on the same day.

From the 96 rats in the main study eighteen pairs of rats which had equal body weights (± 5 gms.) were selected for the paired weight

group. The remainder of the animals in each group were subjected to the endurance training program, while the rest of the rats remained sedentary (c). The rats were forced to run on a motor driven treadmill while progressively increasing the duration and speed until the animals were able to run at 26.8 meters per minute for one hour five days a week.

After eight weeks of training and feeding, the rats were sacrificed. Within each exercise group approximately half the rats were sacrificed 'at rest' (TR) with no exercise on the day of sacrifice, while the other half were exercised to exhaustion (TF) immediately prior to sacrifice.

In the Regeneration Study the pair feeding and paired weight regimens were employed. Within each of these groups one-half the rats underwent surgery to remove the left epididymal fat pad and the other half had both fat pads removed. These groups were again subdivided and one-half subjected to the training program. The selection of subjects for each variable within this study was carried out at random, except to match the body weights of the paired weight group.

This group of rats followed the same feeding and training programs as described above. However, all of the animals did not survive the surgery as well as some being injured while learning to run. The remaining exercise rats, at the time of sacrifice, were then divided into groups which would be sacrificed 'at rest' and 'at fatigue'. Tables 1 and 2 illustrate the groupings of rats for each study.

Table 1

EXPERIMENTAL GROUPS -- DIET AND EXERCISE STUDY

NAME OF GROUP	FEEDING REGIMEN	EXERCISE CONDITION	SACRIFICAL STATE	NUMBER OF RATS PER GROUP
Group I	Ad Libitum	Sedentary	Rest	15
	Ad Libitum	Trained	Rest	6
		Trained	Fatigued	3
Group 2	Pair Fed	Sedentary	Rest	13
	Pair Fed	Trained	Rest	5
		Trained	Fatigued	8
Group 3	Paired Weight	Sedentary	Rest	14
	Paired Weight	Trained	Rest	8
		Trained	Fatigued	6

TABLE 2

EXPERIMENTAL GROUPS -- REGENERATION STUDY

FEEDING REGIMEN	NUMBER OF FAT PADS REMOVED	EXERCISE CONDITION	SACRIFICAL STATE	NUMBER OF RATS PER GROUP
Pair Fed	One	Sedentary	Rest	3
Pair Fed	Two	Sedentary	Rest	4
Pair Fed	One	Trained	Fatigued	3
Pair Fed	Two	Trained	Rest	2
Paired Weight	One	Sedentary	Rest	4
Paired Weight	Two	Sedentary	Rest	2
Paired Weight	Two	Trained	Rest	2
Paired Weight	Two	Trained	Fatigued	1

At the completion of the training and feeding programs (ten weeks) the animals were sacrificed by exsanguination under ether anaesthetic. The entire right and left epididymal fat pads, where present, were surgically removed from the animals. An attempt was made to dissect out all blood vessels and then the pads were weighed on a Fischer Balance. The weights were recorded, the fat pad placed in Rat Ringer's Solution and later frozen in dry ice.

Determination of Fat Cell Size

The preparation of an isolated fixed cell suspension follows exactly the method described by Booth, Booth and Taylor (1973). This procedure involves the collagenase digestions of tissue mince as originally proposed by Rodbell (1964).

A siliconized short-nosed pasteur pipette was then used to transfer the suspension to a vaseline coated glass slide. One drop of methylene blue was added to the slide in an effort to obtain a single layer of dispersed cells. The slide was inspected under the low power of a Vickers microscope and cell sizing was carried out using a previously calibrated ocular micrometer. Seventy five fat cells per slide per fat pad were sized in this manner, and the size distribution was used to calculate mean cell diameter.

Determination of Mean Cell Volume

Mean cell volume was calculated from the diameter data according to the procedure proposed by Di Girolano, Mendlinger & Fertig (1971).

$$V = D^3/6$$

The conversion accounts for the nonlinear transformation.

Determination of Weight of Lipid Per Cell

The calculation for this variable assumes that the measured cell diameter is that of the lipid droplet of the cell, and that the cytoplasmic rim is negligible. The mean lipid weight per cell was calculated for each rat from the mean cell volume by assuming a constant lipid density equal to that of triolein (Hirsch & Gallian, 1968).

$$\overline{M} = \overline{V} \times 0.915 \times 10^{-3} \quad g/cell$$

Determination of the Lipid Content of the Tissue

Lipid content of the fat tissue was determined as a carboxyl ester content by the method of Rapport and Alonzo (1965) after extraction in an isopropanol-heptane system (Dole and Meinertz, 1960). The color reaction was found to be extremely unstable and each testtube had to be treated separately. The lipid concentration was determined from the colourmetric reaction by using a regression equation developed from standard solutions of tripalmitin.

Determination of Cell Number

The total number of cells in the two epididymal fat pads was determined indirectly for each rat from the data on lipid contents of the pad and of the mean cell.

Cell Number = total lipid in pad (mg) x 10^3 ÷ ug lipid/cell.

Statistical Procedures

'T' tests were conducted between the means for cell diameter, lipid content and total number of cells of the right and left epididymal fat pads.

Group means and standard deviations were calculated for all of the variables investigated in the study. A randomized group design two-way analysis of variance was performed on each of the four major variables and a Newman-Keuls multiple comparison test was used to determine the differences between means. The major variables studied were cell diameters, lipid content of the cell, per cent lipid of the total pad and fat cell number.

CHAPTER IV

RESULTS

The means and standard deviations for all the groups in the main study are listed in Table 4. The raw data from which these values are derived can be found in Appendix A. Figures 1 to 4 graphically illustrate comparisons between and within each different group of animals.

The initial computations were 't' tests among groups to determine if there were any differences between the right and left fat pad for the variables under consideration. No significant differences were found, thus the data for both epididymal pads has been pooled throughout the remainder of the study.

The data were subjected to an analysis of variance and significant F ratio's ($p < .001$) were obtained for cell diameter and cell lipid content over the exercise condition (treatment B). Significant B main effects at the 94% confidence level were found for cell number over treatment B. Table 14 in Appendix B summarizes the significant differences between pairs of means as determined by the Newman Keuls comparison procedure. The overall comparison did not yield significant difference for any of the three feeding regimens, (treatment A) nor were there significant interaction effects. Thus the significance table in Appendix B represents those differences found over column means as opposed to cell means.

The sedentary group was found to be significantly different from both groups of trained rats for cell lipid content. The two groups

TABLE 3

FAT CELL SIZE, LIPID CONTENT AND CELL NUMBER FOR LEFT AND RIGHT FAT PADS OF EXERCISED AND CONTROL RATS

PARAMETER	AD LIBITUM GROUP		PAIR FED GROUP		PAIRED WEIGHT GROUP	
	Right	Left	Right	Left	Right	Left
			SEDENTARY			
Fat Cell Size (u)	89.1	85.9	89.0	83.2	66.8	64.6
Pad Lipid Content (%)	73.2	61.2	63.6	63.3	67.2	64.6
Fat Cell Number ($x10^6$)	4.9	4.7	4.4	4.1	8.7	9.6
			TRAINED SACRIFICED AT REST			
Fat Cell Size (u)	74.3	71.6	65.6	67.4	71.6	63.9
Pad Lipid Content (%)	63.4	62.1	51.3	63.4	56.2	50.7
Fat Cell Number ($x10^6$)	4.9	5.3	3.5	5.1	4.0	3.8

TABLE 3 (continued)

PARAMETER	AD LIBITUM GROUP		PAIR FED GROUP		PAIRED WEIGHT GROUP	
	Right	Left	Right	Left	Right	Left
TRAINED SACRIFIED AT FATIGUE						
Fat Cell Size (u)	48.1	51.9	59.6	64.0	62.6	60.7
Pad Lipid Content (%)	69.6	54.7	63.2	60.4	51.7	53.3
Fat Cell Number ($x10^6$)	4.4	4.5	6.1	5.1	6.0	4.3

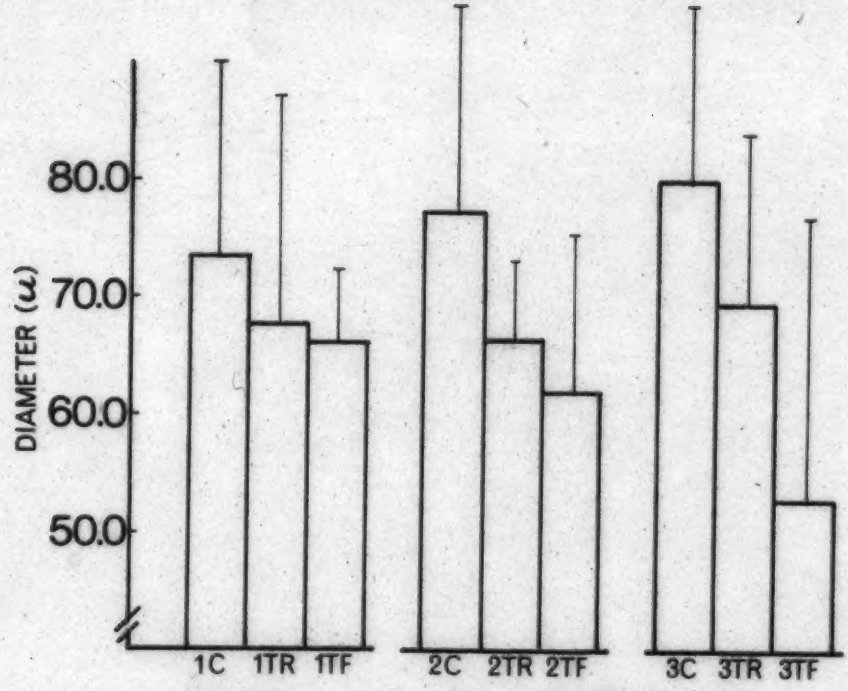

FIGURE 1 MEAN CELL DIAMETERS (u)

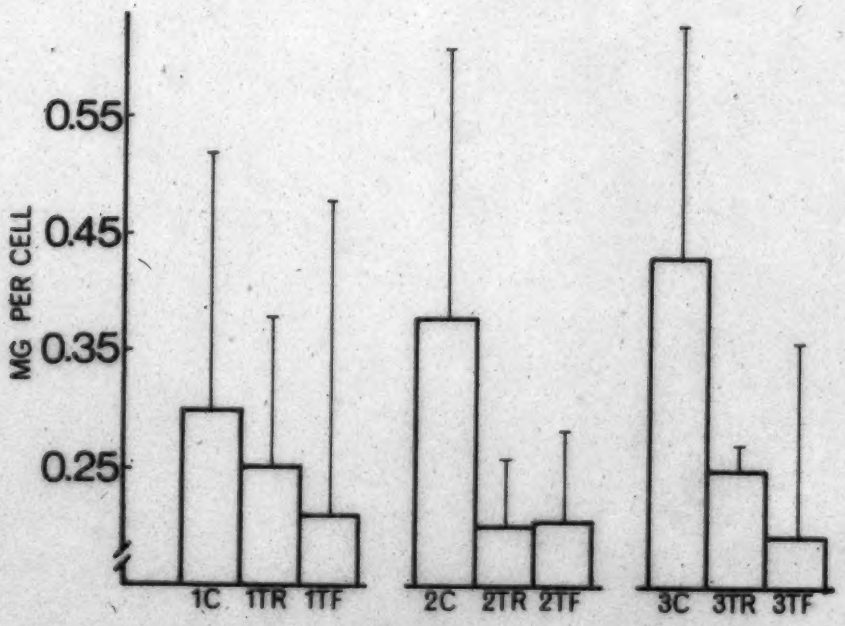

FIGURE 2 LIPID PER CELL (mg)

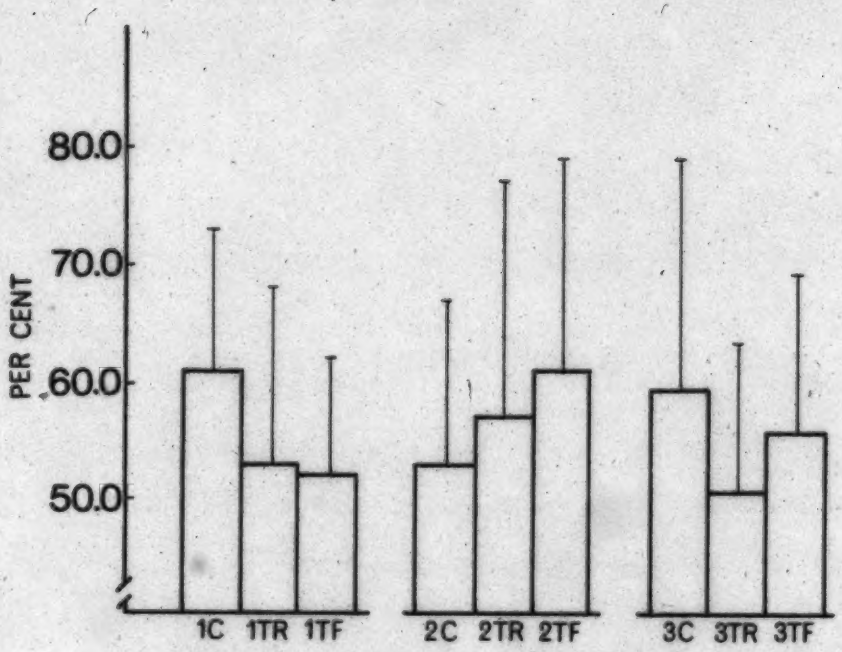

FIGURE 3 PAD LIPID CONTENT (%)

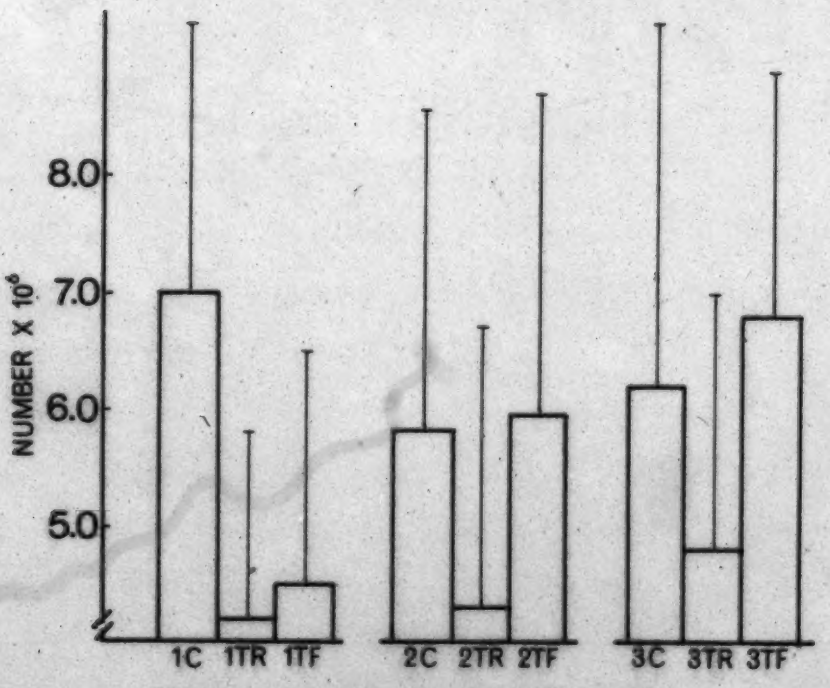

FIGURE 4 FAT CELL NUMBER X 10^6

of trained rats were different only with regard to cell diameter. Per cent lipid per epididymal fat pad was not a significant statistic for any treatment. It will therefore be regarded as unimportant along with the feeding regimen and will not be discussed any further in this chapter.

The overall means for cell diameter were: sedentary 75.7 u, trained sacrificed without previous exercise 68.7 u, and trained sacrificed at fatigue 58.8 u. It was found that the fatigued group differed significantly from both of the other groups at the 95% confidence level. The cell diameters of the sedentary rats tended to be the largest however the difference was not significant between this group and the trained group which were sacrificed 'at rest'.

When considering the amount of lipid per fat cell it was found that the trained rats had significantly less lipid than the non-exercised rats. However, there was no difference within the trained group for those sacrificed under the two exercise conditions. The group means were: sedentary 0.364 mg, trained sacrificed at rest 0.236 mg, and trained sacrificed at fatigue 0.198 mg. Although there seems to be a trend towards a higher lipid content per cell with pair feeding and paired weighing (table 4) this was not found to be statistically significant.

The average cell number per epididymal fat pad over the total sample was 5.62×10^6 cells. For each group the average was as follows: sedentary 6.37×10^6, trained sacrificed at rest 4.48×10^6, and trained sacrificed at fatigue 6.00×10^6. The only variation between groups found to be significant was that between the sedentary rats and the trained rats which were sacrificed 'at rest' (Appendix B, Table 14)

TABLE 4

MEAN CELL DIAMETER, LIPID CONTENT AND CELL NUMBER FOR EXERCISED AND SEDENTARY RATS ON 3 DIFFERENT DIETS

GROUP	MEAN CELL DIAMETER (u)	LIPID PER CELL (mg)	PAD LIPID CONTENT (%)	CELL NUMBER ($x10^6$)
Ad Libitum C (15)[a]	70.36 ± 29.92	.293 ± .17	60.96 ± 12.02	6.98 ± 5.37
Ad Libitum TR (6)	67.65 ± 20.28	.249 ± .13	53.17 ± 14.56[d]	4.15 ± 1.66[f]
Ad Libitum TR (3)	66.22 ± 5.86[bc]	.213 ± .27	52.20 ± 9.78[e]	4.49 ± 2.61
Pair Fed C (13)	77.36 ± 23.01	.375 ± .24	53.48 ± 15.28	5.85 ± 3.66
Pair Fed TR (5)	66.50 ± 6.58	.199 ± .06	57.00 ± 20.65[d]	4.29 ± 2.52[f]
Pair Fed TF (8)	61.98 ± 13.77[bc]	.205 ± .08	61.27 ± 18.81[e]	5.96 ± 3.60
Paired Weight C (14)	79.93 ± 25.08	.429 ± .25	59.50 ± 19.83	6.20 ± 5.35
Paired Weight TR (8)	69.38 ± 14.22	.245 ± .02	50.50 ± 12.68[d]	4.80 ± 2.27[f]
Paired Weight TF (6)	51.89 ± 25.44[bc]	.182 ± .18	55.50 ± 14.13[e]	6.81 ± 2.16

Values are Means ± SD
a - numbers in parentheses are animals per group
b - differences between means for C and TF are significantly different ($p < 0.05$)
c - differences between means for TR and TF are significantly different ($p < 0.05$)
d - differences between means for C and TR are significantly different ($p < 0.01$)
e - differences between means for C and TF are significantly different ($p < 0.05$)
f - differences between means for C and TR are significantly different ($p < 0.05$)

Interesting results were derived in the 'Regeneration Study'. Table 5 summarizes the findings. When one fat pad was removed it regenerated only up to 300 grams which is approximately one-sixth the weight of a normal fat pad. However, when left and right epididymal fat pads were removed greater regeneration was observed. This regrowth of fat was up to 600 gm in the sedentary group and almost complete regrowth was discovered in the exercise groups. The regeneration was also found to be greater in the pair fed than paired weight rats.

The results stated for the regeneration study up to this point are supported by previous literature, however the following results are a product of a small number of rats and cannot be regarded as 'statistically significant'.

The fatigued rats were found to have smaller cells, and lower lipid contents per cell. In general, the cell diameters were smaller for lipidectomized than normal rats, the lipid contents approximately the same and the cell complement increased (except for the case where there was only one surviving subject).

TABLE 5

MEAN CELL DIAMETER, LIPID CONTENT AND CELL NUMBER
OF REGENERATING EPIDIDYMAL FAT PADS
OF EXERCISED AND CONTROL ANIMALS

GROUP	REGENERATED FAT PAD WEIGHT (g)	MEAN CELL DIAMETER (u)	LIPID PER CELL (ug)	PAD LIPID CONTENT (%)	CELL NUMBER ($x10^6$)
Pair Fed C - 1 R*	223.0	65.4	.282	81.2	7.6
Pair Fed C - 2 R	591.3	72.9	-	-	-
Pair Fed TF - 1 R	220.2	48.0	.136	72.4	8.2
Pair Fed TR - 2 R	1860.6	66.5	.248	75.0	7.8
Paired Weight C - 1 R	302.3	69.5	.274	59.1	4.34
Paired Weight C - 2 R	193.6	62.4	.209	38.9	0.25**
Paired Weight TF - 2 R	835.0	39.6	.198	73.5	7.0

Values are Means for animals per group.

* number of fat pads removed
** only one subject in group

CHAPTER V

DISCUSSION

Fat is stored in adipose tissue cells which form depots at various sites in the body. The amount of fat stored in each depot is dependent upon the size and number of its constituent adipocytes. In the present study exercise, by its ability to keep the total body content of fat low (Oscai et al., 1972), significantly reduced the size and number of the epididymal fat cells.

The three feeding patterns which were employed for this study demonstrated no significant differences. Previous research indicates that when food intake is regulated according to the quantity consumed by an exercising rat, the result is restricted feeding of the sedentary paired rat. However, in this study, where there were no differences elicited between any of the feeding patterns, it is obvious that the exercise rat consumed a quantity of food which did not restrict the food intake severely enough to alter the sedentary rat's fat cell characteristics. This result indicates that regular endurance exercise does not significantly depress the appetite as has been previously suggested (Stevenson et al., 1966). Thus the pair feeding and pair weighting programs only ensured that the sedentary rat's diet and weight gain would be regulated to that of the exercising rats which were developing at a rate unaffected by any parameters other than exercise. Thus sedentary rats with diets and body weights regulated to that of the exercise trained rats demonstrate no significant differences in cell size or number. This suggests that exercise was solely responsible for

changes in the fat cells.

Although per cent lipid of the total pad is fundamental to the derivation of total number of fat cells and has been used in previous research as a comparative tool (Booth, Booth and Taylor, 1973), in this study the measurement was subject to such wide variability that no statistically significant data resulted.

The results of this study with regard to fat cell size are very much in agreement with current research. The range of sizes for control and exercise animals in the 18 week old category (Table 4) is slightly smaller than the sizes yielded with methods involving the 'Coulter Counter' (Hirsch and Gallian, 1968). However, this is due to the fact that in the Coulter Counting Method all cells are washed through a 250 u and 25 u mesh nylon filter before they are sized and counted. Thus all cells which are larger than 250 u or smaller than 25 u are excluded from the study. Very few if any cells larger than 250 u are found in normal (as opposed to obese) rats, but many cells smaller than 25 u were observed. Hirsch and Gallian (1968) who proposed the Coulter Counting method were primarily interested in obesity and thus were not greatly concerned with the loss of cells smaller than 25 u. For purposes of the study at hand, however, the smaller mean cell sizes found are still valid measurements and may be critical to the development of a more complete understanding of epididymal fat cell function.

The effects of the exercise training on fat cell size were not clear. The trend, while toward smaller cells was not statistically significant, a point which might merit further exploration by varying the length of the training program, food type or exercise format.

The exhaustive exercise prior to fatigue did produce pronounced and significantly smaller cell diameters.

This was attributed to the fact that the endurance run lasted two to two and one-half hours before exhaustion occurred. At this juncture up to 90% of the energy supply was in the form of FFA liberated from the epididymal fat pads (Issekutz, 1966). This, then, would readily account for the reduction in cell size. The literature supports this finding that the training program alone did not alter cell size. No other experiment on rats over eight weeks of age has produced a permanent alteration in cell size through exercise. In fact the results of Oscai et al. (1972) indicate that in order to produce permanent changes, stress factors must be introduced as early as eight days of age. The remaining possibility is that the training programs must be more severe in order to have greater effects.

The quantity of lipid in each cell proved to be a highly modifiable parameter. The values found agree with other research findings (Grollman and Costello, 1972). It was found that the sedentary animals had a higher lipid content in their mean cell than did either of the exercised group. Such a finding is only reasonable since the exercise rats would constantly be mobilizing FFA from the fat pads thus elevating the turnover rate and increasing the utilization of lipid (Issekutz et al., 1965).

However, the fatigued rats did not possess significantly less lipid which would be expected after two hours of endurance running. The explanation for this may be related to cell size. The cells of the fatigued animals were smaller than the other trained rats, therefore,

if lipid content of the fatigued cells were expressed as a function of the normal, trained size, then the content should be significantly less.

The results for cell number contradict other current findings. Generally, no other training programs instituted on this age group has had significant effects. However, the most common form of exercise used in such studies is swimming which is of a milder nature than the treadmill training. The discrepancy within the results of this study is that the group of exercise rats who were run to fatigue did not have a significantly reduced cell complement. The explanation may be the result of methodological problems. When the epididymal fat pads of the fatigued animals were dissected from the carcas of the rat they were "salmon pink" in colour. The pads of all the other rats were white. This is evidence of increased blood flow to the fat pads and illustrates that the pads were thoroughly perfused with blood after running to fatigue. The slides from the fatigued animals appeared very cluttered indicating that red and/or white corpuscles were probably counted along with fat cells, or the fat cells were extremely fragile after exhaustive mobilization and thus fragmented fat cells were counted. Either of these factors would increase the number and reduce the mean size.

The results of the regeneration study confirm those previously found by Taylor et al. (1971,1972). That is, when two fat pads were removed, more regeneration occurred as opposed to the removal of one fat pad and under conditions of exercise similar and increased regeneration occurred. Running to exhaustion reduced the content of

lipid per cell and the cell size of the regenerated fat pad thus suggesting that the regenerated fat pad is a functional unit and not merely a depot. The number of cells per regenerated fat pad is a highly questionable result in this study. The number is almost twice that of non-lipidectomized rats and may be due to previously suggested difficulties with the method. The slides appeared very cluttered and it is possible that the regenerated fat tissue did not succumb to the collagenase digestion, or the cells were fractured by it. Thus the sizing was extremely difficult and undoubtedly resulted in a large experimental error.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The effects of endurance training, three feeding patterns, exhaustive exercise, and lipidectomy were investigated in 120 male Wistar rats. The rats were eight weeks old at the beginning of the study and were sacrificed between eighteen and twenty-two weeks of age. This was considered to be a developmental period although the critical age beyond which the number of mature fat cells is not subject to change was considered to be fifteen weeks. Epididymal pad lipid content was determined and suspensions of isolated, fixed fat cells were used for microscopic determination of cell diameter. Total cell number was determined indirectly from the lipid contents of mean cell and whole pad.

The pair feeding and paired weighting programs had no significant effects on the fat cells for any parameter used. This points out that the food intake and development of a rat trained from eight to eighteen weeks of age is not significantly different than a sedentary animal.

However exercise training did reduce the total number of fat cells in the fat pad and also reduced the lipid content of the cells. Exhaustive running was found to significantly reduce cell size. Total pad lipid content expressed as a percentage was an uninformative measurement.

Both exercise and bilateral lipidectomy had a positive effect on tissue regeneration. The size and lipid content of such cells were

normal but the number of cells was found to be a questionable measurement and requires further investigation. The effects of fatigue on the regenerated fat cells, reduced size and lipid content, implies that there is a mobilizing function for the regenerated tissue which has previously been considered to serve only as a depot.

Conclusions

Within the limitations of the study it can be stated that; paired weighting and pair feeding had no significant effects on the epididymal fat cells of male Wistar rats, endurance training reduced the total fat cell number and lipid content per cell. Exhaustive running reduced the cell size in both intact and regenerated rat epididymal fat tissue.

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APPENDIX A

RAW DATA

TABLE 6

EPIDIDYMAL FAT PAD DATA IN RATS - FED AD LIBITUM
NON-EXERCISED NOT FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number (x10^6)
109	31.38	.082	80.6	18.72
	31.44	.093	67.9	13.62
110	37.83	.121	83.9	21.02
	49.84	.190	55.3	14.91
111	81.47	.317	60.4	4.14
	73.44	.234	70.1	5.57
112	55.20	.176	75.3	9.49
	79.84	.294	75.8	4.62
113	67.12	.231	41.3	3.98
	71.04	.265	64.8	5.09
114	94.88	.480	55.3	2.94
	114.72	.852	58.5	1.75
115	93.60	.486	61.9	3.51
	-	-	-	-
116	94.08	.472	53.2	3.04
	-	-	-	-
118	57.44	.179	45.4	6.56
	-	-	-	-
119	88.20	.484	69.6	5.01
	-	-	-	-
71	65.60	.218	47.1	4.29
	66.56	.206	70.9	6.09
72	65.92	.217	53.9	4.86
	79.84	.280	66.0	5.16
92	83.44	.356	48.6	2.36
	75.12	.299	65.6	4.58
95	85.84	.482	38.9	2.52
	74.32	.362	-	-
98	42.80	.063	65.6	14.06
	-	-	-	-

TABLE 7

EPIDIDYMAL FAT PAD DATA IN RATS - FED AD LIBITUM
EXERCISED FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
84	58.64	.196	37.92	3.23
	69.20	.230	56.88	3.54
102	66.32	.180	55.30	8.52
	74.00	.242	48.98	5.41
103	62.96	.230	64.89	1.74
	-	-	-	-
NOT FATIGUED AT TIME OF SACRIFICE				
97	58.24	.133	58.46	6.77
	60.88	.170	55.21	5.42
99	77.44	.324	68.63	4.94
	79.45	.380	44.24	2.60
100	81.68	.422	55.30	3.08
	96.32	.416	52.14	2.57
105	57.84	.183	61.16	6.45
	25.28	.023	60.04	-
106	84.03	.347	70.68	3.76
	82.08	.328	30.02	1.67
108	69.04	.208	63.48	4.90
	40.08	.101	22.12	3.50

TABLE 8

EPIDIDYMAL FAT PAD DATA IN RATS - PAIR FED NON-EXERCISED NOT FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
16	101.6	.704	39.3	2.14
	98.6	.682	36.3	1.30
17	80.2	.299	85.1	6.44
	90.7	.425	60.0	3.09
20	85.6	.368	59.03	4.02
	70.3	.221	66.4	7.40
22	102.9	.605	60.3	4.40
	104.3	.632	49.0	3.24
24	94.0	.315	64.8	4.37
	75.4	.495	57.0	6.21
37	82.4	.331	47.7	3.24
	-	-	-	-
38	-	-	-	-
	53.0	.115	43.2	4.01
40	31.4	.051	26.3	10.58
	-	-	-	-
45	77.2	.310	52.0	6.48
	-	-	-	-
68	103.2	.851	41.6	1.36
	96.8	.728	77.8	3.25
69	44.5	.105	53.6	13.87
	47.4	.132	49.3	9.25
93	82.5	.320	53.9	4.94
	66.6	.238	77.8	9.85

TABLE 9

EPIDIDYMAL FAT PAD DATA IN RATS - PAIR FED EXERCISED
FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
4	33.20	-	-	-
	41.60	.114	65.00	7.58
7	69.68	.204	42.85	3.13
	69.92	.260	42.66	2.18
8	70.72	.231	84.04	13.89
	84.00	.404	80.54	5.06
10	60.64	.235	49.82	2.34
	39.68	.073	58.46	8.10
29	59.92	.150	48.52	3.60
	63.84	.179	36.34	4.22
57	59.68	.163	92.2	10.18
	62.24	.175	53.2	4.80
123	62.80	.196	52.1	4.73
	67.20	.265	56.9	4.00
126	65.44	.173	96.38	12.26
	81.12	.315	66.36	3.61
	NOT FATIGUED AT TIME OF SACRIFICE			
3	71.44	.246	12.15	1.07
	62.00	.215	54.00	5.08
11	74.40	.293	54.92	2.59
	67.28	.228	52.14	3.09
33	63.92	.165	71.40	7.30
	75.04	.245	64.09	4.41
125	58.08	.117	80.58	8.36
	59.84	.132	69.52	2.40

TABLE 10

EPIDIDYMAL FAT PAD DATA IN RATS - PAIRED WEIGHT NON-EXERCISED NOT FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number (x10⁶)
13	99.40	.566	78.2	4.41
	93.60	.469	77.4	5.05
14	61.84	.263	69.6	6.97
	67.20	.622	77.4	4.48
15	65.28	.228	95.0	8.81
	64.49	.219	77.4	7.04
18	45.52	.129	58.7	20.95
	-	-	-	-
19	27.44	.069	28.7	18.52
	42.40	-	-	-
21	115.00	.827	75.8	4.51
	120.50	.968	41.1	1.99
39	98.4	.538	60.2	2.48
	89.4	.388	39.5	2.04
43	-	-	-	-
	-	-	-	-
44	-	-	-	-
	-	-	-	-
46	82.40	.319	76.6	7.35
	-	-	-	-
47	47.28	.093	75.9	15.27
	-	-	-	-
48	64.64	.059	13.6	3.95
	-	-	-	-
67	95.5	.556	40.8	2.14
	101.3	.658	56.9	2.14
	99.4	.560	59.8	2.54
	98.7	.540	71.5	2.91

TABLE 10 (continued)

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
94	109.6	.739	50.6	2.06
	-	-	-	-
96	73.3	.256	67.5	7.09
	75.8	.291	45.7	4.06

TABLE 11

EPIDIDYMAL FAT PAD DATA IN RATS - PAIRED WEIGHT
EXERCISED FATIGUED AT TIME OF SACRIFICE

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
1	44.8	.015	69.6	7.04
	-	.239	-	-
5	19.9	.013	57.6	-
	50.2	.115	69.4	9.77
6	19.9	-	-	-
	27.7	-	-	-
12	47.1	.151	49.4	7.90
	54.9	.173	34.8	4.79
25	44.4	.081	28.4	5.89
	39.9	.066	79.0	-
36	86.6	.431	58.6	5.06
	109.2	.611	56.9	2.99
81	68.0	.186	57.6	6.94
	62.0	.142	53.6	9.45
	NOT FATIGUED AT TIME OF SACRIFICE			
2	51.2	.122	73.0	16.0
	75.0	.334	42.0	2.99
9	86.6	.412	85.9	5.06
	97.1	.603	49.9	1.70
30	73.4	.250	59.7	4.74
	74.4	.281	56.9	3.22
32	89.1	.414	44.9	2.80
	78.2	.269	80.6	7.36
35	48.7	.086	59.6	9.87
	55.0	.107	66.4	7.62
58	74.2	.269	49.8	1.39
	65.2	.185	66.1	6.48

TABLE 11 (continued)

Rat No.	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
79	51.7	.099	48.5	5.80
	63.5	.162	54.5	4.20
122	64.7	.206	53.9	5.51
	62.1	.196	69.5	3.66

TABLE 12

REGENERATION OF EPIDIDYMAL FAT PADS IN RATS - PAIRED WEIGHT NON-EXERCISED

Rat No.	Fat Pad Weight (mg)	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
	ONE PAD REMOVED - NOT FATIGUED AT TIME OF SACRIFICE				
61	2062.3	80.5	.351	32.7	1.93
	106.5	53.4	.162	-	-
62	2383.8	75.7	.273	81.21	7.09
	142.2	35.7	.084	-	-
63	2521.4	79.5	.294	44.4	3.80
	156.2	53.1	.158	-	-
87	3595.6	102.6	.617	77.9	4.54
	199.7	75.4	.265	-	-
	TWO PADS REMOVED - NOT FATIGUED AT TIME OF SACRIFICE				
65	193.7	72.7	.302	38.9	.25
	263.4	68.2	.257	-	-
88	120.2	57.04	.159	-	-
	196.8	51.65	.119	-	-
	EXERCISED				
	TWO PADS REMOVED - NOT FATIGUED AT TIME OF SACRIFICE				
77	830.2	44.6	.074	3.65	.41
	1476.2	45.8	.079	7.35	1.37
78	775.8	84.1	.441	60.48	1.06
	187.8	-	-	71.48	2.84
	TWO PADS REMOVED - FATIGUED AT TIME OF SACRIFICE				
52	1072.0	39.9	.069	77.42	12.39
	151.1	39.3	.062	69.52	1.69

TABLE 13

REGENERATION OF EPIDIDYMAL FAT PADS IN RATS -
PAIR FED - NON EXERCISED

Rat No.	Fat Pad Weight (mg)	Cell Diameter (u)	Cell Lipid (mg lipid/cell)	Pad Lipid (%)	Cell Number ($x10^6$)
	ONE PAD REMOVED - NOT FATIGUED AT TIME OF SACRIFICE				
51	134.2	50.9	-	-	-
	-	-	-	-	-
85	2120.0	51.2	.150	-	-
	103.8	40.4	.096	67.9	9.60
86	3160.8	97.4	.512	94.5	5.83
	342.2	86.9	.371		
	TWO PADS REMOVED - NOT FATIGUED AT TIME OF SACRIFICE				
64	259.1	72.9	-	-	-
	220.0	74.1	-	-	-
66	353.8	66.9	-	-	-
	393.7	63.4	-	-	-
89	340.8	96.2	-	-	-
	112.2	78.8	-	-	-
90	451.5	69.1	-	-	-
	282.0	62.3	-	-	-
	EXERCISED				
	ONE PAD REMOVED - FATIGUED AT TIME OF SACRIFICE				
73	2824.2	39.12	.084	74.56	24.77
	220.4	46.2	.129	-	-
74	2340.2	31.6	.062	62.7	24.04
	220.4	75.2	.158	-	-
75	1717.6	75.2	.242	79.0	5.61
	220.0	48.7	.152	-	-

APPENDIX B

SIGNIFICANCE TABLE

TABLE 14

SIGNIFICANT DIFFERENCES BETWEEN MEANS AS DETERMINED BY NEWMAN KEULS COMPARISON

GROUP	MEAN CELL DIAMETER	CELL LIPID CONTENT	CELL NUMBER
C vs TR	-	$p < 0.01$	$p < 0.05$
C vs TF	$p < 0.05$	$p < 0.01$	-
TR vs TF	$p < 0.05$	-	-